Transgenerational inheritance of DNA hypomethylation in *Daphnia magna* in response to salinity stress

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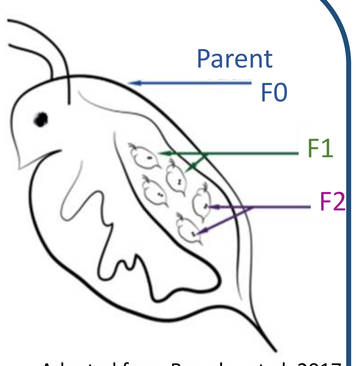
Introduction

- Epigenetic mechanisms play an important role in environmental stress response and regulation.
- Epigenetic marks can be inherited through successive generations, yet few studies have shown persisting stress-induced transgenerational effects.
- Daphnia is a parthenogenetic model and key species in freshwater ecosystems, widely considered among regulatory frameworks.

Aim

To explore the potential for transgenerational inheritance (maintained up to unexposed F3) of DNA methylation patterns in *Daphnia* after F0 exposure to salinity and the underlying molecular mechanisms.

Material & Methods

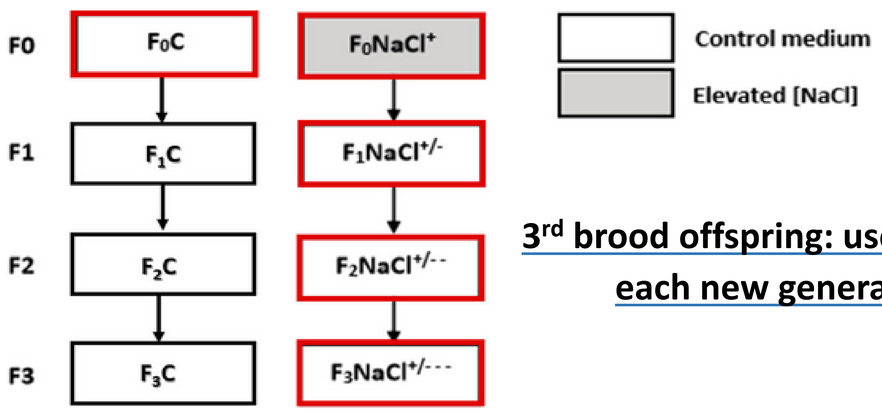


Adapted from Brander et al. 2017

In addition to F0, the somatic and/or germline cells of developing embryos can also be directly exposed to the parental stressor

➔ **So… DNA methylation patterns were monitored until F3 generation to spot "true" transgenerational effects**

Six monoclonal cultures of 70 neonates
(**0 g/L NaCl (x 3)** and **4.1 g/L NaCl (x 3)** in ASTM medium)



3rd brood offspring: used to start each new generation

+ and - indicate the history of each culture regarding exposure to NaCl (+) and maintenance in clean medium (–)

Red boxes: treatments from which DNA was extracted

DNA extraction

MasterPure Complete DNA and RNA Purification Kit
(Epicenter, Madison, WI, USA)

↓

Reduced Representation Bisulfite Sequencing

↓

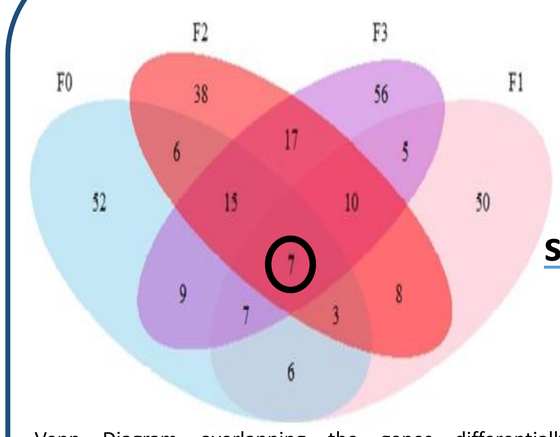
Bioinformatic and Statistical analysis

FastQC; Trim Galore!; Bismark; R software

Life history traits

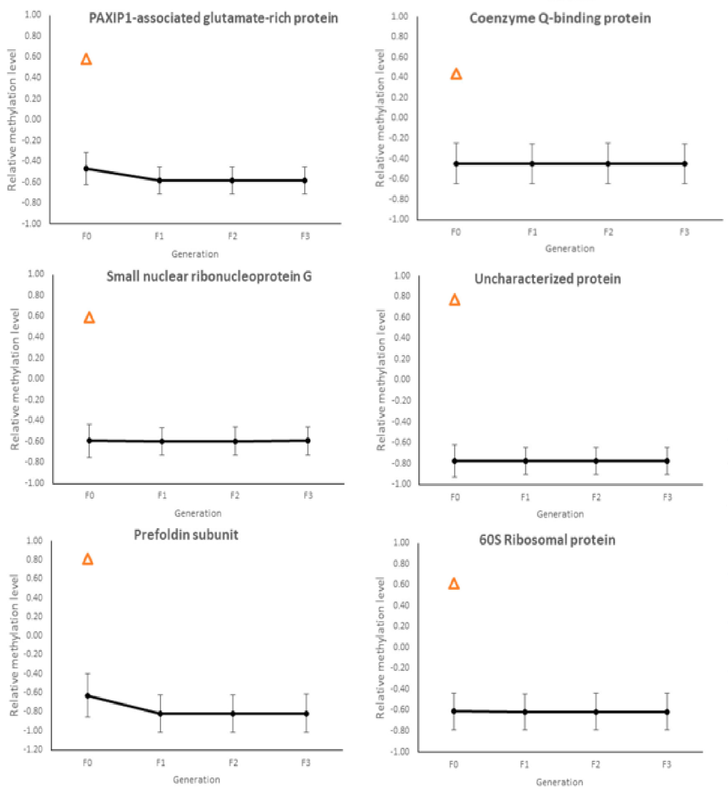
age at first reproduction,
net reproductive rate,
per capita rate of population increase

Results



Venn Diagram overlapping the genes differentially methylated between NaCl treatments and the control through the different generations (p<0.05)

7 genes significantly differentially methylated across the four generations (F0–F1–F2–F3)

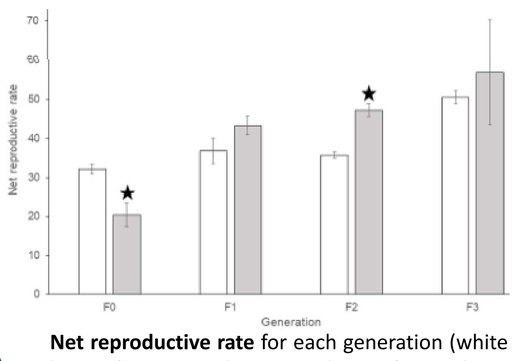


Differences in methylation (**in black**) between the control and F0-F3 salinity treatment
Control treatment methylation is plotted in **orange**

Higher methylation levels in the control than in the exposed and following non-exposed generations

Hypomethylation of **six genes** with important roles in response to environmental change:

- **DNA damage repair,**
- **cytoskeleton organization**
- **protein synthesis**



Net reproductive rate for each generation (white bars for control, grey bars for salinity treatments). ★significant effects (p<0.05)

No main effects of NaCl across all generations for the *per capita* rate of population increase, age at first reproduction or net reproductive rate

Concluding remarks

- The exposure of a single *D. magna* generation to high levels of salinity (F0) provoked **specific methylation patterns** that were **transferred to the three non-exposed generations** (F1,F2,F3).
  - **Epigenetic changes** seem to be particularly **targeted** to **genes** involved in coping with **general cellular stress responses**.
- **No life history costs** seemed to be associated with the **maintenance of hypomethylation** across the unexposed generations.

Epigenetic marks can be useful indicators of past or historic pollution in *Daphnia*

Literature cited

Brander SM, Biales AD, Connon RE (2017) The Role of Epigenomics in Aquatic Toxicology. Environ Toxicol Chem. 36:2565–2573. doi: 10.1002/etc.393

Acknowledgements

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